LIFE-HISTORY STUDIES IN SCLEROTINIA

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(WITH PLATE 3)

For several years past a species of *Sclerotinia* has been observed by the writer in a certain stretch of woods in the upper end of Van Cortlandt Park, New York City, on the rootstocks of wild geranium. Although this has been seen abundantly in this particular region, it has not been detected by us in other localities where the wild geranium grows. The apothecia usually appear early in the spring about the latter part of April or early in May and disappear early in June. A search of the records showed no species of *Sclerotinia* listed for this host, so that the writer was uncertain whether this represented an undescribed species or some old species on a new host. It did not appear, however, to agree well with any described species, and it was finally decided to publish it in order to bring it to the attention of mycologists. Before doing this it was thought advisable to locate the conidial stage, if possible, in order to make the description more complete, and during the spring of 1917 this work was undertaken.

From our knowledge of other species of *Sclerotinia*, it was thought that the conidial stage might be located as a parasite on the leaves or other living tissue of the host. A careful search of the region in which the fungus occurred on various occasions showed nothing on the living plants which could be suspected of being the conidial stage of this fungus. A collection of infected rootstocks, however, which had been brought into the laboratory and placed in a moist chamber after a few days showed a most luxuriant growth of a species of *Botrytis*. This appeared in dense tufts not only on the rootstocks and rootlets, but even covered over the outside of the apothecia like a coat of fur. While none of the *Botrytis* was apparent on the plants when brought into the laboratory, it invariably appeared within a few days on the roots

and rootstocks of those plants infected with *Sclerotinia*, while similar rootstocks from regions where the *Sclerotinia* was absent failed in every case to develop this type of *Botrytis*. The conidiophores often appeared in dense tufts, these often springing from minute black sclerotium-like bodies, although the latter were not always evident. Thinking that this fungus might be an omnivorous saprophyte, the rootstocks of other kinds of plants from the same region were placed in moist chambers, but failed to produce this fungus. From these rough observations it was suspected that the *Botrytis* might have some connection with the *Sclerotinia*.

It was noted that the ascospores were always in excellent germinating condition when brought into the laboratory, and it was decided to attempt to culture out the fungus. One of us (Horne), who happened to be working at the New York Botanical Garden at this time, kindly offered to culture the fungus and the following experiments were conducted by him.

CULTURE EXPERIMENTS

Crude cultures were made by touching the tufts of *Botrytis* spores, as shown in the accompanying plate, on the small roots of wild geranium with a sterile needle and then bringing the needle into contact with an autoclaved potato plug placed in a test tube. A vigorous fungus grew promptly, developing in somewhat the same way as *Botrytis vulgaris*, but readily distinguished from that species on detailed examination. A few days later a crude culture was made by touching the top of an apothecium of the *Sclerotinia* of wild geranium with a sterile needle and with this inoculating a drop of sterile water on a sterilized slide. The *Sclerotinia* spores were abundant and no *Botrytis* spores were observed in the drop on examination with the 16 mm. objective. A transfer was made from this drop to a potato plug, as with the *Botrytis* inoculation. After somewhat more than one week, these cultures were examined and both were producing the characteristic *Botrytis* spores and microconidia.

On May 20, pure cultures of the *Sclerotinia* of wild geranium were made as follows: a dried herbarium specimen of the *Sclerotinia* collected in Van Cortlandt Park during the present season was moistened by placing a small drop of sterilized water on the upper

surface, using a loop needle and sometimes slightly rubbing the surface of the hymenium with the loop. Some of the material was then transferred to a drop of sterile water on a sterilized slide. Poured plate cultures were made in the usual way, being inoculated directly from the drop prepared on the slide, using prune-juice agar and Shear's cornmeal agar. Before making the poured plates, the drop used for inoculation was carefully inspected with the 16 mm. objective and no *Botrytis* spores were seen in it, in fact no spores were observed except those of the *Sclerotinia*.

After about twenty hours the agar plates were inverted and a number of germinating spores were marked. Only the colonies of mycelium clearly arising from one spore and well separated were marked. While the original spore had become considerably swollen and not recognizable with absolute certainty in some cases, it appeared from the figure of the mycelium that the *Sclerotinia* spore had given rise to the growth in every case marked. Later in the same day five of these colonies were transferred to slant tubes of prune agar. The following day six more plantings were made from separate colonies growing from marked single ascospores. By this time the colonies had become very complex and were plainly visible. Three days from the making of the poured plates the colonies were confluent and vigorous and the characteristic *Botrytis* spores had commenced to be formed. Apparently all of the colonies originating from the ascospores gave rise to the *Botrytis* spores and there were no contaminations, all of the colonies being of the same sort.

Of the single-spore transfers to prune-juice agar, three of those made on the first day failed to grow, presumably the young mycelium had been caught on the needle used in the transfer, since some had not been found on the slant after making the transfer. The remaining eight single-spore cultures developed very uniformly and all produced abundant *Botrytis* spores.

On June 24, plantings were made from each of the eight pure cultures on prune-agar slants to sterilized geranium rootstocks and to sterilized potato plugs. On the potato plugs the growth was identical with that originally secured from crude plantings of the spores of *Botrytis* and *Sclerotinia*. After four days, *Botrytis* spores could be seen with a lens in nearly all of the cultures and

yellowish sclerotia were beginning to form on some of the geranium rootstocks, but no distinct sclerotia were observed on any of these or older potato cultures. Although *Botrytis* spores appeared in all of these, they were much more abundant on the rootstocks than on the potato plugs. On the rootstocks the spores were so abundant as to be evident to the naked eye, and the masses were very similar to those obtained on infected rootstocks brought from the field and placed in moist chambers, as shown in the accompanying plate. Check experiments were kept and these failed to show any evidence of the *Botrytis*.

The infected rootstocks will be kept with the hope of securing mature apothecia, but these will probably not appear until spring, as is the case in nature, and it is too soon to predict what the result of this study will be. However, the production of *Botrytis* directly from the ascospores of the *Sclerotinia* confirms field observations on the connection of the two fungi. The production of apothecia would add still more interest to the investigation.

Sclerotinia (**Stromatinia**) **Geranii** sp. nov.

Conidial stage (*Botrytis*) occurring on the roots and rootlets of the host, being especially abundant when left in moist chamber for a few days and even developing on the outside of the apothecia, usually appearing in tufts and often springing from minute sclerotium-like bodies, although the latter are not always present, dark brown in mass at maturity; conidiophores reaching a length of 1 mm. or more and a diameter of 10–15 μ, pale brown, sparingly septate and branched, the conidia borne in rather large masses like bunches of grapes; conidia subglobose or pyriform, the small end representing the point of attachment, reaching a diameter of 10 μ or rarely as large as 12 μ, slightly longer than broad, at first smooth, becoming quite strongly roughened, pale brown with transmitted light.

Apothecia springing from the partially decayed rootstocks in clusters of variable numbers, stipitate, shallow-cupshaped or subdiscoid, reaching a diameter of 1 cm. or rarely larger, pale-brown externally; hymenium concave or nearly plane, a little darker than the outside of the apothecium; stem reaching a diameter of 2 mm. and often reaching a length of several cm., though often short and occasionally almost wanting, the length varying with the depth to which the rootstocks of the host are buried; asci cylindric or subcylindric, 8-spored, reaching a length of 120–140 μ

and a diameter of 8–10 μ; ascospores hyaline, ellipsoid or almond-shaped, 4–5 $\mu \times$ 12 μ, usually containing two very small oil-drops. [PLATE 3.]

On the rootstocks of wild geranium (*Geranium maculatum*). The type collected in woods in the upper end of Van Cortlandt Park, New York City, May, 1917.

The subgenus *Stromatinia* has been raised to the rank of a genus by Boudier, although it is not commonly regarded as such. If the genus *Stromatinia* is considered distinct from *Sclerotinia* our plant would be designated as **Stromatinia Geranii.**

DESCRIPTION OF PLATE 3

FIG. 1. Photographs of the *Botrytis* stage on the underground parts of wild geranium, natural size, with drawings of sporophores and conidia enlarged.

FIG. 2. Photograph of apothecia, natural size, with drawing of ascus with spores and germinating ascospores, enlarged.

All drawings made with the aid of the camera lucida to a common scale using a one-inch eye-piece and a one-sixth objective.